Chronic graft-versus-host disease (CGVHD) is more frequent following peripheral blood stem cell (PBSCT) than bone marrow transplantation (BMT). However, the responsiveness of the disease to immune-suppression using either stem cell source has not been well validated. We compared the time to discontinuation of immune-suppression between PBSCT and BMT in 184 patients with CGVHD after sibling donor transplantation. Of 717 sibling donor transplants (1990–2004), the cumulative incidence of CGVHD was 25% (95% CI 22–28%) overall and was more frequent after PBSCT [39% (95% CI 32–46%) versus BMT 17% (95% CI 14–20%), $p < 0.01$]. The incidence of discontinuation of immune-suppression (DIS) was 9% (95% CI 5–13%) at 1 year, and 67% (95% CI 52–82%) by 5 years [DIS at 1 year: BMT 15% (95% CI 7–23%) versus PBSCT 5% (95% CI 1–9%); and at 2 years: 38% (95% CI 26–50%) versus 32% (95% CI 22–42%); however both approached 67% by 5 years]. The multivariate analysis was restricted to adult patients who received myeloablative conditioning (n = 130, 63 BMT, 67 PBSCT) due to interaction between pediatric age group and stem cell source (17/19 pediatric patients received BMT); and conditioning regimen and stem cell source (35/37 receiving non myeloablative conditioning regimen also received a PBSCT). PBSC recipients, those with progressive onset of CGVHD and thrombocytopenia (<100,000/μl) at diagnosis of CGVHD had lower rates of DIS than BM recipients (Table). After a median follow-up of 33 months, a trend towards superior overall survival at 5 years was seen in the BMT cohort [5 year survival: BMT 65% (95% CI 53–75%) versus PBSCT 58% (95% CI 45–69%) $p = 0.07$]. Following sibling donor transplantation, CGVHD is more frequent and more resistant to immune-suppressive therapy using PBSC vs. BM. PBSC recipients were significantly more likely to require prolonged immune-suppression and extended morbidity from CGVHD. Careful attention to this serious later morbidity and mortality should guide clinical decisions about the use of either BM or PBSC sources for sibling donor transplantation.

Time to Discontinuation of Immune Suppression	Hazard Ratio (95% C.I.) (>1 = more frequent/ quicker DIS)	P
Stem Cell Source		
Marrow	1.0	<0.01
PBSC	0.5 (0.3–0.8)	
Onset of CGVHD		
Quiescent/Denovo	1.0	0.01
Progressive	0.5 (0.3–0.7)	
No. of Organs involved with GVHD		
1	1.0	
2	0.8 (0.4–1.4)	0.29
3+	0.7 (0.3–1.3)	0.21
Thrombocytopenia		
<100,000/μL	1.0	<0.01
≥100,000/μL	2.0 (1.3–3.3)	
Overall Survival [Kaplan-Meier estimate (95% CI)], p = 0.07		
	Marrow	PBSC
At 2 years	77% (66–84%)	70% (60–78%)
At 5 years	65% (53–75%)	58% (45–69%)

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EFFICIENT AND SELECTIVE PREVENTION OF GRAFT-VERSUS-HOST DISEASE BY ANTIGEN-SPECIFIC TGFβ-INDUCED REGULATORY T CELLS

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Naturally occurring regulatory T cells (nTregs) suppress the development of graft-versus-host disease and may spare graft-versus-tumor effect. As nTreg is a rare cell population in a healthy individual, using *in vitro* expanded nTregs is a common strategy to test their therapeutic potential in hematopoietic cell transplantation (HCT). However, the concern of *in vitro* expanded nTregs may include their stability of Foxp3 expression and suppressive function, survival *in vivo*, and non-selective suppression of pre-activated nTregs. In this study, we have used an alternative strategy to generate antigen-specific, induced Tregs (iTregs). CD4+CD25- cells from OT-II TCR transgenic, *foxp3/gfp* knock-in mice were induced to express Foxp3 by incubating with OVA peptide in the presence of TGFβ. CD4+GFP+ cells were purified by sorting and used as iTregs while CD4+GFP- cells as controls. Their ability to prevent GVHD was tested in a lethally irradiated murine BMT model: B6 → (B6 × bm12)F1. In order to evaluate the specificity of iTregs, OVA-expressing or non-expressing (B6 × bm12)F1 recipients were compared side by side. We found that iTregs (CD4+GFP+) completely prevented GVHD lethality in OVA+ recipients at a Treg:Teff ratio of 1:5. The efficacy of these antigen-specific iTregs were significantly higher than polyclonal nTregs from B6 donors as they could only partially prevent GVHD and prolong recipient survival even at a 1:1 ratio. In contrast, iTregs failed to prevent GVHD in OVA- recipients. As controls, CD4+GFP- cells had no effect on GVHD development in OVA- recipients, and even exacerbated GVHD in OVA+ recipients compared to B6 CD4+ effector cells alone. These results reveal the therapeutic potential of antigen-specific iTregs to prevent GVHD efficiently and selectively.

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GENERATION OF T_{REG}-LIKE CELLS FROM CD4+CD25- T CELLS VIA EPIGENETIC MODIFICATION USING A DEMETHYLATING AGENT DECITABINE

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Naturally-occurring regulatory T cells (nT_{reg}) contribute to the maintenance of self-tolerance and have been reported to suppress autoimmunity and GvHD in mouse models. Major obstacles for their routine use in human clinical trials include the low number of T_{reg}, low efficiencies of purification and severe limitations in *ex vivo* expansion without downregulating the expression of *Foxp3* that is the master gene of T_{reg}. Recent identification of demethylated CpG islands within the *Foxp3* locus only in T_{reg} lead us to investigate whether an FDA-approved demethylating agent, Decitabine, could be used to enhance expression of Foxp3 via an epigenetic effect and convert CD4+CD25- T cells into T_{reg}. We incubated human CD4+CD25-FOXP3- T cells with anti-CD3/CD28 beads and hIL-2, followed by Decitabine treatment. Real time RT-PCR demonstrated levels of mRNA for *FOXP3* that were comparable to that seen in bead-activated nT_{reg}. This resulted in increased expression at the protein level in 60% of treated cells. Decitabine also induced Foxp3 expression in 80% of murine CD4+CD25- T cells incubated with anti-CD3/CD28 beads and hIL-2, followed by Decitabine treatment. The upregulation of Foxp3 by Decitabine treatment was further validated with GFP expression in CD4+CD25- T cells from Foxp3-ires-GFP KI mice. The CD4+CD25- T cells expanded approximately two fold comparable to 13–20 fold of the number of naïve nT_{reg}. These Decitabine-treated CD4+CD25- T cells (dcT) showed suppressor function in MLR. We next tested whether these cells were able to suppress GvHD in an allogeneic BMT model. Mice that were transplanted with dcT showed significantly higher survival rate and maintained their weight better. The T_{reg} surface markers, such as GITR, CTLA4, and CD25 were upregulated in the dcT. We also found that GzmA and GzmB were upregulated. Using Prf 1 KO and GzmB KO mice, we found that the suppressor function of the dcT is partially dependent on Prf 1, but not on GzmB. A transwell experiment demonstrated that the suppressor function of dcT is cell-contact dependent. The gene expression profiles were also compared between dcT, naïve nT_{reg}, and bead-activated nT_{reg}. We found that dcT expressed high level of *CTLA-4*, *GITR*, *CD25*, and *FR4*, which have been known to be upregulated in nT_{reg}. In summary, Decitabine-treatment of CD4+CD25- T cells enhanced Foxp3 expression. These dcT showed suppressor function in both *in vitro* and *in vivo* and shared part of gene signature of nT_{reg}.